

Progress Report for National Aeronautics and Space Administration Grant  
NSG-375 for the period November 1, 1964 through April 30, 1965.

Principal Investigators: Nathan O. Kaplan, Professor and Chairman,  
 Graduate Dept. of Biochemistry, Brandeis University  
 Lawrence Levine, Professor, Grad. Dept. of  
 Biochemistry, Brandeis University  
 Lawrence Grossman, Assoc. Professor, Grad.  
 Dept. of Biochemistry, Brandeis University.

Evolutionary Studies -- Lactic dehydrogenases (LDH's) from over one hundred species, representing all the vertebrate classes were studied. The main properties measured were: susceptibility to inhibition by high pyruvate concentration, electrophoretic mobility in starch gel, and temperature stability.

Most species were found to have two major types of LDH:  $H_4$  LDH, characterized by its occurrence in the heart and its susceptibility to pyruvate inhibition, and  $M_4$  LDH, characterized by its occurrence in skeletal muscle and its relative lack of susceptibility to pyruvate inhibition.

The following species seemed to contain only one type of LDH: flatfishes (order Heterosomata), the lamprey Petromyzon marinus, and the cephalochordate Amphioxus. The enzyme of flatfishes and the lamprey resembles the  $M_4$  enzyme of other vertebrates. It might be possible that the single LDH found in Amphioxus and the lamprey represents an early evolutionary stage, predating the evolutionary appearance of the two types of LDH found in higher vertebrates.

The  $H_4$  enzyme was more thermostable than the  $M_4$  enzyme in many species but the reverse was true in various species of turtles and amphibians.

The  $H_4$  enzyme of many species moved faster toward the anode than did the  $M_4$  enzyme but the two enzymes had identical mobilities in some bird species and the  $M_4$  enzyme of the cod (Gadus callarias) moved faster than the  $H_4$  enzyme.

Some fishes and amphibians seemed to lack the hybrid LDH's,  $H_3M$ ,  $H_2M_2$ , and  $HM_3$ .

Under standard electrophoretic conditions, the  $H_4$  enzyme of various species moved the following distances toward the anode: most fishes, 4-8 cm; most amphibians, 4-10 cm; most reptiles 5-6 cm; most birds, 1-3 cm; most mammals, 12-15 cm.

No great systematic change in  $H_4$  mobility seemed to have taken place during such early stages of vertebrate evolution as the evolution of the fish classes, the evolution of amphibians from fishes, or the evolution of reptiles from amphibians. Structural changes in the  $H_4$

N65-25590

1

FACILITY FORM 602

(ACCESSION NUMBER)	(THRU)
3	1
(PAGES)	(CODE)
CR 63246	04
(NASA CR OR TMX OR AD NUMBER)	(CATEGORY)

GR0.	---
CR571	---
HC.	100
MF.	50

enzyme leading to a reduction in mobility when birds evolved from reptiles and to a rise in mobility when mammals evolved from reptiles seemed to have occurred.

Under standard conditions, the inactivation temperature for the  $H_4$  enzyme was not far from 60-65° for most species of fishes, amphibians, turtles (anapsid reptiles) and mammals tested and not far from 75-80° for all the diapsid reptiles and most of the bird orders tested.

It was suggested that a structural change leading to a great rise in thermostability of the  $H_4$  enzyme took place when diapsid reptiles evolved from anapsid reptiles.

The  $M_4$  enzyme of most species tested had a very low mobility under the standard electrophoretic conditions.

The inactivation temperature for the  $M_4$  enzyme was determined for relatively few species; most of the values fell in the range 50-60°.

The possible use of electrophoretic and thermostability data for solving taxonomic and phylogenetic problems was examined.

Immunochemical Studies -- A protein unique to nervous tissue was recently discovered in a number of vertebrate species (rat, rabbit, guinea pig, chicken, catfish, alligator, beef, monkey, and human), and the protein from beef brain was isolated and purified. The protein, an unusually acidic component of the brain-extract soluble fraction, was found in roughly equal concentrations in gray and white matter and spinal cord. With antibodies to this acidic protein, it has been shown that, in contrast to most vertebrate proteins, the structure of the acidic protein exhibits little evolutionary variation.

Since antibodies to the purified beef brain acidic protein (BBAP) could not be obtained by direct immunization, a procedure was applied to make the BBAP immunogenic by complexing it with methylated bovine serum albumin (MBSA), a method which had been used successfully to obtain antibodies to negatively charged molecules such as DNA and polysaccharides and polyribonucleotides. Antibodies to BBAP antigen were successfully produced with MBSA - BBAP complex as the immunogen, and the antiserum was shown to be immunochemically homogeneous in double diffusion tests in agar with crude beef brain extract and purified BBAP as antigens.

In double diffusion experiments with this antiserum, a precipitating band of identity was found with brain extracts of several species. The complement (C') fixation curves obtained with antiserum to the purified BBAP and brain extracts of several animals were also similar. The lack of species differences in this brain acidic protein is striking. Only one other protein is known to resemble the brain acidic protein in this respect -- lens protein.

The serological activity of the rabbit brain extract is of further interest. Although the serum of the immunized rabbit contains antibodies

directed toward the animal's own brain protein, the rabbit has shown no signs of illness. Either the blood - brain barrier is sufficient to shield the brain from effects of the antibody, or the acidic protein is located intracellularly -- a site which cannot be reached by the circulating antibody. The cellular location of the protein is currently under study in experiments with labeled antibody.

Effect of Irradiation on Nucleic Acids -- We have just completed a series of experiments in which the ultraviolet(UV) photoproducts of cytosine act as uracil in RNA polymerase templating systems. We are presently studying the effects of other mutagens whose chemical products are very analogous to the UV photoproducts. It appears as though similar modifications produce identical templating changes. We are presently modifying chemically the cytosine moieties of polycytidylic acid and also the nucleoside triphosphate substrates. These experiments will serve to indicate what structural lines are necessary for changes in recognition systems as well as to show the site at which mutagenesis can occur.